Enrichment of Ferulate Phytosterol Esters from Corn Fiber Oil Using Supercritical Fluid Extraction and Chromatography

Sir:

We previously reported on the methodology of supercritical fluid extraction (SFE) coupled with supercritical fluid fractionation (SFF) (1,2) for the enrichment of high-value nutraceutical components from agricultural products. The enrichment of these compounds is necessary because of the low levels of these products in the natural matrix. This SFE/SFF methodology is attractive to the processor and the consumer because it employs environmentally benign carbon dioxide (CO_2) and ethanol (EtOH), which enjoys Generally Regarded As Safe (GRAS) status in the United States for use in food processing. We now report on applying the SFE/SFF technique to a new matrix, corn fiber oil, for the enrichment of ferulate phytosterol esters (FPE), which recently have been shown to have cholesterol-lowering activity (3,4).

In prior work (2), we described the SFE/SFF of corn bran oil using an amino-propyl bonded silica for the fractionation/enrichment of FPE. The same methodology has now been applied to corn fiber oil, which has a starting FPE concentration greater than corn bran oil. Moreau *et al.* (5,6) noted that the oil obtained from corn fiber contains higher FPE levels than corn bran oil. They indicated that corn fiber oil may contain FPE up to 6.75 wt%, whereas corn bran oil contains FPE up to 1.5 wt%.

A supply of corn fiber was acquired from the Archer Daniels Midland Company (Decatur, IL). It was not ground before extraction with supercritical carbon dioxide ($SC\text{-}CO_2$) at 34.5 MPa and 40°C. Multiple extractions were performed on a home-built extractor to yield a sufficient supply of corn fiber oil for SFF studies. The collected extracts were combined into one vial and stored at 4°C until use. The average yield of oil was 0.56 wt% of the fiber; however, if the corn fiber had been ground, more oil could have been extracted by SFE (and hexane) as shown by Moreau *et al.* (5). Regardless, the FPE content of this corn fiber oil was higher than that reported by Moreau *et al.* (5); it contained FPE at a 23.12 wt% level. In addition, the corn fiber oil in our study contained triglycerides at ~49 wt%, whereas the corn fiber oil of Moreau *et al.* consisted of 80–90 wt% triglycerides.

As in our earlier work (2), multiple SFF experiments were performed using the same sorbent bed (5 grams of an amino-propyl sorbent) to prevent the generation of large amounts of waste sorbent. The sorbent bed was reused and reconditioned as previously described. The effect of maximum sample load was not investigated as the fractionation was performed on ~0.4 g samples of the corn fiber oil. The SFF runs were accomplished using an Isco (Lincoln, NE) model SFX 3560 automated extractor and analyzed as previously described (2). The extraction/fractionation procedure is detailed in Table 1. Again, the first fraction removed most of the triglyceride-based oil and the fourth and fifth fractions (which were combined for analysis) contained the FPE. From here on, "final fraction" refers to the fourth and fifth fractions combined.

For the six SFF runs, the average mass balance showed an approximate total recovery of 99.2%. The mass recovered during the sorbent reconditioning runs was higher than our previous SFF study involving corn bran oil (2). The present work yielded 3.15 mg on the average for the reconditioning step. This translated into an average mass recovery of 0.8%, and when combined with the SFF average mass balance, a recovery of 100% is attained. Thus, carryover from run to run does not seem to be problematic.

The bulk of the mass was collected in the first and final fractions. On average, the first fraction contained 51.9% of the mass and the final fraction contained 46.6%. These average percent recoveries translate into 211.0 mg and 189.3 mg, respectively. The mass of extract collected in the final fraction shows a 500% higher level than in the corn bran oil SFF study where the final fraction averaged 37.7 mg (2).

In agreement with prior work (2), the first fraction contained the majority of the fatty acid phytosterol esters and triglycerides. This fraction was approximately 15 wt% esters and 85 wt% triglycerides. The final fraction mainly consisted of ferulate phytosterol esters, free fatty acids, free sterols, and minor amounts of 1,3- and 1,2-diglycerides. On average, the FPE made up 53.36 wt% of this fraction, while the starting corn fiber oil was 23.12 wt% FPE. Thus, the FPE were enriched over twofold. This enrichment to >53 wt% translates to a fraction containing 101 mg of FPE. Comparing this to the earlier study where FPE made up ~5.5 mg of the enriched fraction, corn fiber oil yields 18.4 times more FPE than corn bran oil. This greater percentage of enrichment along with the larger mass of the fraction yields a considerable enrichment of FPE from corn fiber oil as compared with corn bran oil.

Table 2 contains the SFF recovery data. The reported average recoveries of the fatty acid phytosterol esters and the FPE indicate total quantitative recovery within experimental

TABLE 1
Parameters for the Supercritical Fluid Fractionation of Corn Fiber Oil

Fraction	Pressure (MPa)	Temperature (°C)	Time (min)	Flow rate (mL/min)	Solvent (vol %)
1	69.0	80	60	2	CO_2
2	34.5	40	60	2	1% EtOH/CO_2
3	34.5	40	60	2	2% EtOH/CO_2
4	34.5	40	90	2	10% EtOH/CO_2
5	34.5	40	90	2	10% EtOH/CO_2

TABLE 2
Percent Recoveries from Supercritical Fluid Fractionation of Corn Fiber Oil

Individual runs	Mass	FASE[a]	TG[b]	FPE[c]
1	99.66	101.14	99.01	104.36
2	100.92	104.32	92.35	105.89
3	100.56	105.10	95.09	99.69
4	97.30	101.54	95.76	96.50
5	99.86	97.78	93.60	104.39
6	96.88	94.70	91.82	102.36
Average recoveries	99.20	100.76	94.61	102.20

[a]Fatty acid phytosterol esters
[b]Triglycerides
[c]Ferulate-phytosterol esters

variation. The triglyceride recoveries are a little low (~95%) and may be due to some hydrolytic action indicated by the presence of 1,2-diglycerides and free fatty acids. In contrast to previous data (2), the free sterols in this study exhibited lower recoveries (~74 wt%), and this warrants further investigation.

In this study, we have extended a two-step process of SFE coupled with SFF to enrich and fractionate ferulate phytosterol esters from corn fiber oil. The described process provides an alternative to conventional phytosterol extraction, which requires specialized equipment such as fractional or molecular distillation units and their attendant high energy requirements (7). This SFE/SFF method is now being scaled up to a larger preparative mode to include an examination of the effect of sample load.

Although this SFE/SFF technique has only been applied to vegetable oils, it has potential to enrich/fractionate tall oil, which contains free phytosterols and ferulate phytosterol esters. A margarine containing FPE (mainly sitostanol), Benecol, has been produced and patented to Rasio Company (8) and licensed to McNeil (a division of Johnson and Johnson) in the United States.

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